UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: March 15, 2001

MEMORANDUM

Subject: **THIOPHANATE-METHYL. CASE # 2680**. Revised Toxicology Chapter for the Reregistration Eligibility Decision Document.

Tox. Chem. No.:	375A
PC Code No.:	102001
DP Barcode No.:	D272850
Submission No.:	S571296

From: Deborah Smegal, M.P.H., Toxicologist
Reregistration Branch III
Health Effects Division (7509C)
and
Linnea J. Hansen, Ph.D.
Toxicology Branch I
Health Effects Division (7509C)

Through: Catherine Eiden, Branch Senior Scientist
Reregistration Branch III
Health Effects Division (7509C)

To: Deanna Scher, Product Reviewer
Reregistration Branch II
Special Review and Reregistration Division (7508W)

Attached please find the Toxicology Chapter for incorporation into the RED Document for the fungicide thiophanate-methyl. This chapter incorporates the "error-only' comments received from the registrant during Phase 1 of the Tolerance Reassessment Advisory Committee (TRAC) process. This document summarizes the toxicology studies submitted to support reregistration of thiophanate-methyl and the toxicity endpoints selected for risk assessment.

1.0 HAZARD CHARACTERIZATION

1.1 Hazard Profile

1.1.1 Adequacy of Toxicology Database/Data Gaps: At this time, the toxicology database for thiophanate-methyl is incomplete. Additional information is needed to upgrade the rat gavage developmental toxicity study (MRID 00106090) to acceptable (see subsection 1.1.4 on developmental toxicity, below, for details). The Hazard Identification Assessment Review Committee (HIARC, meeting of April 8, 1999) requested that rat acute and subchronic neurotoxicity screening studies and a developmental neurotoxicity study be submitted (see subsections 1.1.5 on neurotoxicity and 1.1.7 on structure-activity considerations, and section 1.3, FQPA Considerations, below, for rationales). The Cancer Assessment Review Committee (CARC, April 28, 1999 meeting) requested submission of the following additional genotoxicity studies: a preincubation *Salmonella typhimurium* mammalian microsome gene mutation assay, a mouse lymphoma L5178 cell forward gene mutation assay with colony sizing and a mouse *in vivo* bone marrow assay with antikinetochore staining. In addition, the metabolite 2-aminobenzimidazole metabolite should be tested at minimum in the *S. typhimurium* mammalian microsome gene mutation assay (see Genotoxicity, subsection 1.1.6, below, for rationales).

The quality of the currently available acceptable toxicity studies on thiophanate-methyl is considered high.

1.1.2 Subchronic/Chronic Systemic Toxicity: Subchronic and chronic toxicity studies on thiophanate-methyl are summarized below in Table 1. The liver and thyroid are the primary target organs of thiophanate-methyl in several species following subchronic or chronic dietary exposure. In the Fischer-344 rat subchronic toxicity study, thyroid and liver enlargement, hepatocellular hypertrophy and thyroid hypertrophy/hyperplasia were observed, although alterations in thyroid hormone levels were not reported. In the chronic toxicity/carcinogenicity study on Fischer-344 rats, thyroid and liver were enlarged and alterations in circulating thyroid hormones (increased TSH; decreased T3/T4) were observed. Serum cholesterol was also increased. Microscopically, liver hypertrophy, lipofuscin pigmentation, focal fatty degeneration and necrosis were observed in males and hypertrophy and lipofuscin deposition in females. Thyroid hypertrophy and hyperplasia were seen in both sexes. In the beagle dog, similar thyroid and liver effects and related clinical chemistry alterations were also observed with subchronic or chronic exposure. Serum alkaline phosphatase was also increased following chronic exposure. In the 18-month CD-1 mouse carcinogenicity study, liver enlargement and hypertrophy, and enlarged thyroid and hypertrophy/hyperplasia, were also reported. However, thyroid effects were less pronounced than in the rat or dog, with enlargement and hypertrophy/hyperplasia and sporadic circulating hormone alterations observed only at high dose levels (>1000 mg/kg/day). The effects observed in the thyroid are consistent with disruption of the thyroid-pituitary homeostasis, but additional information is considered necessary to sufficiently support this mechanism (see subsection 1.1.8 on mechanistic studies, below).

In addition to liver and thyroid effects, thiophanate-methyl also appeared to cause mild anemia at the higher dose levels in rats, dogs and mice following subchronic or chronic exposure. In rats, thiophanate-methyl caused toxicity to the kidney and increased urinary protein (males), lipofuscin pigmentation and increased severity of nephropathy were reported following chronic

administration. An increase in systemic calcification was observed in males and to a lesser extent in females and was probably secondary to hyperparathyroidism. Decreased body weight/weight gain was observed in both sexes. Male rats appeared to be more sensitive than females based on greater severity of effects and high mortality at the highest dose tested (6000 ppm or 280.6 mg/kg/day, males and 334.7 mg/kg/day, females). Beagle dogs also showed decreased body weight. In the 1 year dog study, transient tremors at the highest dose tested (HDT) (200 mg/kg/day) were also observed. In the mouse carcinogenicity study, increased heart weight (females) and incidence of atrial thrombosis were observed.

Thiophanate-methyl is a carbamate but only limited data are available on its potential to inhibit cholinesterase (ChE). As a class of compounds, thiocarbamates do not produce consistent cholinesterase inhibition patterns. In the rat subchronic toxicity study, serum cholinesterase activity was increased in males by 22-38% relative to controls but decreased in females by 25-28% at $\geq$293.2 mg/kg/day. In the rat chronic toxicity/carcinogenicity study, males showed increases in serum ChE at 280.6 mg/kg/day (HDT) at 6 and 12 months (41-42%) whereas at 24 months, it was decreased (-38%). ChE activity in females was slightly decreased (18-35%) at 6 and 12 months at $\geq$63.5 mg/kg/day. RBC and brain ChE activities were not evaluated. ChE was not measured in the subchronic or chronic dog studies.

Thiophanate-methyl administered dermally to rabbits over a period of 21 days (5 days/week, 6 hrs/day) caused decreased food consumption in females at 300 and 1000 mg/kg/day and in males at 1000 mg/kg/day. Because this decrease was reported in both sexes and a dose-response was observed in females, it is considered treatment-related although no other signs of toxicity were observed. Comparison of this dermal LOAEL with an oral LOAEL (maternal toxicity, rabbit developmental toxicity study) suggests that thiophanate-methyl is poorly absorbed into the skin. Dermal absorption was estimated at about 7% of the applied dose.

The only inhalation toxicity study submitted was a 14-day inhalation toxicity study on a formulation containing 5.2% thiophanate-methyl. Local pulmonary effects were observed at the LOAEL of 0.0151 mg/L and decreased body weights at the HDT. However, in addition to testing a formulation and not the technical a.i., this study did not evaluate all of the standard parameters (e.g., clinical chemistry, hematology, organ weights, complete gross/microscopic tissue evaluation) and therefore does not provide adequate information on toxicity via the inhalation route.

Table 1: Subchronic and chronic toxicity studies of thiophanate-methyl			
Guideline	**Study**	**Doses (mg/kg/day except as noted)**	**Results**
870.3100 [82-1(a)]	90-Day Dietary Toxicity Study in Rats MRID No. 42001701, 42533802 Date: 1990 Acceptable-guideline	♂ 0, 13.9, 155.0, 293.2, 426.9 or 564.7 ♀ 0, 15.7, 173.4, 323.0, 478.8 or 647.3 Tech., 96.55% a.i.	NOAEL = 15.7 mg/kg/day LOAEL = 155.0 mg/kg/day, based on anemia, increased serum cholesterol and calcium (males), increased liver and thyroid weights, increased kidney (males) weight and increased incidence of thyroid hyperplasia/hypertrophy, liver swelling and lipofuscin deposition, and glomerulonephrosis (males) were observed. At higher dose levels, effects included increased serum cholinesterase (males), decreased thymus weight (females), increased incidence of glomerulonephritis (females) and fatty degeneration of the adrenal cortex were also reported.
870.3150 [82-1(b)]	90-Day Oral (Capsule) Toxicity Study in Beagle Dogs MRID No. 41982203 Date: 1992 Acceptable-guideline	0, 50, 200 or 800 in gelatin capsules (HDT lowered to 400 on day 50 due to excessive toxicity) Tech., 96.55% a.i.	NOAEL = 50 mg/kg/day LOAEL = 200 mg/kg/day, based on thin/dehydrated appearance, tarry stools, decreased body weight/weight gain, decreased food consumption, slight anemia, increased serum cholesterol, decreased serum T3/T4 (females), increased liver and thyroid weights, thyroid follicular cell hypertrophy and hyperplasia, hypoplasia/atrophy of the prostate, thymic involution/atrophy (males) and depletion of spleen lymphoid cells. At 800/400 mg/kg/day, mortality (1 male), increased platelet count were also observed.
870.3200 [82-3]	21-Day Dermal Toxicity Study in New Zealand White Rabbits MRID No. 42110801 Date: 1991 Acceptable-guideline	0, 100, 300 or 1000, moistened with water (5 days/week, 6 hrs/day) Tech., 96.55% a.i.	Systemic toxicity NOAEL = 100 mg/kg/day Systemic toxicity LOAEL = 300 mg/kg/day, based on decreased food consumption in females. At 1000 mg/kg/day, consumption also decreased in males. Slight dermal irritation was observed at all dose levels.
870.3465 [82-4]	14-Day Inhalation Toxicity Study in HSD:(SD) Rats MRID No. 42527601 Date: 1992 Unacceptable-nonguideline	0.0, 0.00514, 0.0151 or 0.247 mg/L Tech., 5.2% a.i. (Tops® 5 formulation)	NOAEL = 0.00514 mg/L LOAEL = 0.0151 mg/L, based on increased incidence of alveolar macrophages, pneumonocyte hyperplasia of the lung and nonsuppurative alveolitis. At 0.247 mg/L, decreased body weight gain (females) and increased incidence of lung microgranulomas (both sexes) were also observed.

Table 1: Subchronic and chronic toxicity studies of thiophanate-methyl			
Guideline	**Study**	**Doses (mg/kg/day except as noted)**	**Results**
870.4100 [83-1b]	1-Year Oral (Capsule) Study in Beagle Dogs MRID No. 42311801 Date: 1992 Acceptable-guideline	0, 8, 40 or 200 in gelatin capsules Tech., 96.55% a.i.	NOAEL = 8 mg/kg/day LOAEL = 40 mg/kg/day, based on decreased body weight/weight gain, markedly increased serum TSH (1 male) and decreased T4 (males), increased serum cholesterol (males), increased abs/rel thyroid weights (both sexes) and thyroid follicular cell hypertrophy (females). At 200 mg/kg/day, tremors in all dogs 2-4 hrs postdosing (most on day 1; sporadically through day 17), slight anemia, increased serum alkaline phosphatase and cholesterol, increased relative liver weight, thyroid follicular cell hypertrophy in males and hyperplasia (both sexes) were also observed.
870.4200b [83-2b]	18-Month Dietary Carcinogenicity Study in CD-1 Mice MRID No. 42607701 Date: 1992 Acceptable-guideline	♂ 0, 23.7, 98.6, 467.6 or 1078.8 mg/kg/day; ♀ 0, 28.7, 123.3, 557.9 or 1329.4 mg/kg/day Tech., 95.93% and 96.55% a.i.	Systemic toxicity NOAEL = 23.7 mg/kg/day in females and 98.6 mg/kg/day in males Systemic toxicity LOAEL = 123.3 mg/kg/day for females based on hepatocellular hypertrophy, and 467.6 mg/kg/day in males. At ≥123.3 mg/kg/day, decreased body weights, sporadic effects on circulating T4 and TSH, increased thyroid and liver weights, increased heart weight (females), increased hepatocellular hypertrophy and increased atrial thrombosis were also observed. At the HDT, mortality was increased in both sexes. Increased incidence of hepatocellular adenomas in males at ≥467.6 mg/kg/day (control to high dose, 9%, 17%, 17%, 42% and 57%[a]) and in females at ≥123.3 mg/kg/day (0%, 0%, 8%, 24% and 56%[a]). Both sexes showed significant increasing trends and pairwise increases at the highest two dose levels. a incidence/statistical analysis calculated by HED (memorandum from L. Brunsman to N. McCarroll and L. Hansen, dated 3/16/00)

Table 1: Subchronic and chronic toxicity studies of thiophanate-methyl			
Guideline	**Study**	**Doses (mg/kg/day except as noted)**	**Results**
870.4300 [83-5]	24-Month Dietary Chronic Toxicity/ Carcinogenicity Study in F-344 Rats MRID No. 42896601 Date: 1993 Acceptable-guideline	♂ 0, 3.3, 8.8, 54.4 or 280.6 ♀ 0, 3.8, 10.2, 63.5 or 334.7 Tech., 96.55% a.i.	NOAEL = 8.8 mg/kg/day LOAEL = 54.4 mg/kg/day, based on decreased body weight/weight gain (males; marginal in females), decreased food efficiency (males; marginal in females), sporadic effects on circulating T3/T4 and TSH, increased serum cholesterol and creatinine, decreased serum cholinesterase in females, increased liver, thyroid and kidney weights, liver hypertrophy and lipofuscin accumulation, thyroid hypertrophy and hyperplasia and lipofuscin accumulation in the kidney. At≥280.6 mg/kg/day, excessive mortality in males (2/50 survivors at termination), decreased body weight/weight gain in females, mild anemia, increased urinary protein, hyperparathyroidism (primarily in males), systemic calcification, increased severity of nephropathy and increased severity of liver and thyroid effects were also observed. The HDT was considered excessive in males. Increased incidence of thyroid follicular cell adenoma in males (control to high dose, 2%, 0%, 0%, 6% and 27%[a]) and females (0%, 0%, 0%, 2% and 4%[a]). Significantly increased trend in both sexes; pairwise incidence in males at high dose. Follicular cell carcinomas also observed in high dose males at high dose (11% vs. 0% all other doses; significant trend and pairwise comparison). Combined incidence significantly increased at high dose (2%, 0%, 0%, 6% and 32%) with positive increasing trend. a incidence/statistical analysis calculated by HED (L. Brunsman in memo to N. McCarroll/L. Hansen dated 3/16/00)

Subchronic Inhalation Toxicity in Rats. In the 14 day inhalation study (MRID 42527601), thiophanate-methyl, 5.2% a.i. was administered to 3 groups of 10 HSD:(SD) rats/sex/dose by whole body exposure at concentrations of 0.0, 0.05745, 0.6181 or 1.0326 mg/L (nominal concentrations); 0.0, 0.00514, 0.0151, or 0.0247 mg/L (analytical concentrations) for 6 hours per day for a total of 14 days. After the treatment period, the animals were retained and observed for an additional 14 days. No treatment-related effects were observed at 0.00514 mg/L. At 0.0151 mg/L and above there were increases in alveolar macrophages and pneumonocyte hyperplasia of the lung in both sexes (no statistical analysis available). Nonsuppurative alveolitis were also observed in these groups as well. At 0.0247 mg/L, there were a few microgranulomas in the lungs of both sexes and there was a decrease in bodyweight gain in females (76% after 14 days exposure; 86% after additional 14 day observation period). **The LOAEL is 0.0151 mg/L, based on increases in alveolar macrophages and pneumonocytic hyperplasia in both sexes and decreases in body weight gain in females. The NOAEL is 0.00514 mg/L.** This subacute inhalation toxicity study is supplementary because it was not intended to satisfy a guideline requirement. The study is not upgradable.

Chronic Toxicity in Dog. In a chronic oral toxicity study (MRID 42311801), 4 beagle dogs/sex/dose group were administered thiophanate-methyl (tech., 96.55% a.i.) daily for 1 year by gelatin capsule at doses of 0, 8, 40 or 200 mg/kg/day.

At 40 mg/kg/day, decreased mean body weight/weight gain (compared to controls at termination, -7%/-19%, males and -6%/-19%, females; not statistically significant), decreased mean serum T4 levels in males at 6 months (-46%) and markedly increased TSH in 1 male at 6 and 12 months (approximately 2-fold over pretest), increased serum cholesterol in males at 6 and 12 months (+47% and +30%; latter not significant), increased absolute/relative thyroid weights (+33%/+42%, males and +28%/+10%, females; not statistically significant) and thyroid follicular epithelial cell hypertrophy (2/4 females) were observed. At 200 mg/kg/day, tremors (mostly moderate in all dogs; observed 2-4 hrs post-dosing between days 1-17), slightly decreased Hgb, Hct and RBC in males at 6 and 12 months (-13% to -14% below controls), increased serum ALP at 6 and 12 months (+100% and +300%, males and +47% and +82%, females; not significant in females) and cholesterol at 6 and 12 months (+51% and +42%, males; latter not significant and +93% and +76%, females), increased relative liver weights (+46%, males and +35%, females) and thyroid follicular epithelial cell hyperplasia (1 male and 1 female) were observed. Decreases in body weight/weight gain, increases in thyroid weight and follicular cell hypertrophy and effects on thyroid hormones were more pronounced than at 40 mg/kg/day. Slight decreases in serum A/G ratio, Ca^{++}, K^{+} and phosphate in males were reported but not considered toxicologically significant. There were no treatment-related effects on survival, ophthalmologic parameters or urinalysis. **The LOAEL is 40 mg/kg/day, based on decreased body weight/weight gain and thyroid effects. The NOAEL is 8 mg/kg/day.**

This study is classified **Acceptable (§83-1b)** and satisfies the guideline requirement for a chronic oral toxicity study in the dog.

1.1.3 Carcinogenicity: The results of the rat and mouse carcinogenicity studies are summarized above in Table 1. Thiophanate-methyl caused a dose-related increase in the incidence of thyroid follicular cell tumors in male and female F-344 rats at the highest 2 dose levels ($\geq$54.4 mg/kg/day). In males, a positive increasing trend and a pairwise increase in incidence of adenomas, carcinomas and combined adenomas/carcinomas at the HDT were observed (see Table 1 for percent incidence). In females, the incidence of adenomas was lower and showed a significant increasing trend but no pairwise increase. No carcinomas were observed. In both sexes, the incidence was increased above available historical control values; however, this data was not from the study lab or from the same supplier within 2-3 years of the study conduct. In CD-1 mice, statistically significant, dose-dependent increases in hepatocellular adenomas were observed in males at the highest 2 dose levels ($\geq$467.6 mg/kg/day) and also in females at $\geq$123.3 mg/kg/day (see Table 1 for percent incidence). A significant increasing trend was also observed in both sexes. The combined incidence of adenoma and carcinoma was also increased in males, but the incidence of carcinomas alone was not increased. The incidence of adenomas were above the available historical control values for studies from the same lab and for the same strain from the supplier, some within 2-3 years of the conduct of this study.

Thiophanate-methyl is classified as "likely to be carcinogenic to humans" by the HED Cancer Assessment Review Committee (CARC) on April 28, 1999. A Q_1^* of 1.38×10^{-2} $(mg/kg/day)^{-1}$ was assigned based on the dose-dependent increases in liver tumors in male mice (quantitative risk

assessment memorandum from L. Brunsman to N. McCarroll and L. Hansen dated March 16, 2000). The thyroid tumors in rats were also considered treatment-related because a dose-dependent increase was observed in both sexes (in males, toxicity at the HDT was excessive based on high mortality but the tumors were nonetheless considered treatment-related). Although evidence supporting a threshold mechanism for thyroid tumor induction based on disruption of thyroid-pituitary homeostasis was submitted, the CARC determined that additional information (e.g., demonstration of reversibility of treatment-induced thyroid hormonal alterations and morphological changes after cessation of treatment, additional genotoxicity studies) was required to adequately demonstrate this mechanism. Special mechanistic studies submitted in support of this mechanism are described below (subsection 1.1.8).

A. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Executive Summary: In a 2-year feeding/oncogenicity study (MRID 42896601), thiophanate methyl was administered in the diet to 60 male and 60 female F344 rats/group at 0, 75, 200, 1200 or 6000 ppm. After week 52, 10 rats/sex/dose were sacrificed, except only five 6000 ppm males were sacrificed because 8 males died from non-treatment related injury at weeks 11 and 12. The mean compound consumption for the study was 0, 3.3, 8.8, 54.4 and 280.6 mg/kg/day for males and 0, 3.8, 10.2, 63.5 and 334.7 mg/kg/day for females.

Rats fed 75 and 200 ppm had no significant treatment-related toxic effects. Male rats fed 1200 ppm and 6000 ppm had significantly decreased mean body weights and net weight gains at the end of the study. The mean weight of 1200 ppm males was 84% of controls ($p<0.001$), and the net gain was 79% of controls ($p<0.001$), whereas the two 6000 ppm males which survived to week 104 had a mean weight 73% of controls and net weight gain 63% of controls. Female rats had significant body weight changes only in the 6000 ppm dose group, the mean weight was 78% ($p<0.001$) and the mean net gain was 69% ($p<0.001$) of controls at the end of the study. The 1200 and 6000 ppm males and females had decreased food efficiency. Food efficiency in rats fed 1200 ppm was reduced to 78% and 88% of controls in males and females, respectively, while in 6000 ppm rats, the efficiencies were lowered to 65% and 71% in males and females. There was a treatment-related decrease in survival in only the 6000 ppm group males (2/55 survivors vs. 37/50 controls, $p<0.001$); the marginal increase in mortality ($p<0.05$) in the 200 ppm group males appeared spurious. Other male groups and all female dose groups were unaffected. Non-neoplastic pathological changes were observed primarily in 1200 and 6000 ppm rats in the liver (dose-related weight increase and hepatocellular hypertrophy), kidney (surface changes, dose-related increase in weight and severity of nephropathy), and thyroid (dose-related weight increase, follicular cell hypertrophy and hyperplasia, and T_3 and T_4 hormone level decreases). The levels of thyroid stimulating hormone (TSH) were elevated, though pituitary weights were unchanged. **A LOAEL of 1200 ppm was identified for both male (54.4 mg/kg/day) and female (63.5 mg/kg/day) rats, based on treatment-related effects in the liver, kidneys, and thyroid and decreased body weight in males. The corresponding NOAEL was 200 ppm in both sexes of rats (corresponding to 8.8 mg/kg/day for males and 10.2 mg/kg/day for females), based on lack of significant toxic effects at this dose**.

The toxic effects observed in the thyroid in 1200 and 6000 ppm male and female rats were accompanied by a dose-related increase in the incidence of follicular cell adenoma (males: 1/50, 0/48, 0/50, 3/50, 12/55 and females: 0/50, 0/49, 0/50, 1/50, 2/50 for doses of 0, 75, 200, 1200, and 6000 ppm, respectively). The increase was statistically significant ($p<0.01$) only in males at 6000

ppm, a dose which was shown to exceed the maximum tolerated by males by the high mortality it caused. The thyroid adenoma was likely a secondary effect of the thyroid-pituitary hormonal imbalance induced by chronic compound treatment. The increased incidences of neoplasms in the spleen and adrenal medulla were not dose-related and were of uncertain biological significance (spleen mononuclear cell leukemia in 75 and 200 ppm males and in 75, 200, and 1200 ppm females and adrenal medulla pheochromocytoma in 75, 200, and 1200 ppm males). There were also several neoplasms which were statistically elevated but incidental to treatment (skin papilloma in 75 ppm males, pituitary adenoma in 200 ppm males and mammary gland fibroadenoma in 1200 ppm females). Based on the significant depressions in mean body weights and mean net body weight gains in the rats, it appears that the maximum tolerated dose (MTD) was achieved in the study for both males (1200 ppm or 54.4 mg/kg/day) and females (6000 ppm or 334.7 mg/kg/day).

Discussion of Tumor Data: At 6000 ppm, the incidence of thyroid follicular cell adenoma was increased in males (from control to high dose, 2%, 0%, 0%, 6%, 24%) and females (from control to high dose, 0%, 0%, 0%, 2% and 6%). The increase was statistically significant only in males at 6000 ppm ($p<0.01$), which was considered an excessive dose (see below). Other tumors were observed at increased incidence above controls but did not show a dose-response and were not considered treatment-related. These included (1) increased incidence of adrenal medulla pheochromocytoma at 75, 200 and 1200 ppm in males and (2) increased incidence of mononuclear cell leukemia in the spleen in males at 75 and 200 ppm in males and 75, 200 and 1200 ppm in females (incidence of this leukemia in specific organs does not reflect total incidence within a group).

Adequacy of the Dose Levels Tested: In males at the highest dose tested (6000 ppm), dosing was considered excessive and the MTD was exceeded based on excessive mortality. Dosing was considered adequate at 1200 ppm based on thyroid, liver and kidney effects in both sexes and decreased body weight/weight gain in males. The Cancer Assessment Review Committee (CARC) was divided as to whether the MTD was exceeded in females at 6000 ppm (report dated August 24, 1999, HED Doc no. 013688). Nevertheless, the CARC concluded that the increased incidence of thyroid tumors at 6000 ppm was biologically significant since a dose-response was observed in both sexes.

B. Carcinogenicity Study in Mice

Executive Summary: In a dietary carcinogenicity study (MRID 42607701), thiophanate-methyl (tech., 95.93 to 96.55% a.i.) was administered daily to 50 CD-1 mice/sex/dose at concentrations of 0, 150, 640, 3000 or 7000 ppm for 18 months (equivalent to average daily intakes of 0, 23.7, 98.6, 467.6 or 1078.8 mg/kg/day, males and 0, 28.7, 123.3, 557.9 or 1329.4 mg/kg/day, females). An additional 10 mice/sex/dose were administered these dose levels and sacrificed at 39 weeks.

At 640 ppm, increased incidence of hepatocellular hypertrophy was observed in females (8% vs. 0% affected, controls). At 3000 ppm, slightly decreased mean body weights in males, primarily during the middle of the study (<8% below controls; gain -12% below controls at week 53), transiently increased TSH (week 39, +100% above controls), increased abs/rel thyroid weights in males (+52%/+64% above controls, week 39 only), increased abs/rel liver weights (+20 to +26% above controls, males and females), increased incidence of hepatocellular hypertrophy in males and females (25% affected vs. 10%, controls and 10% vs. 0%, controls, respectively) and increased incidence of

atrial thrombosis in females (35% vs. 0%, controls) were observed. At 7000 ppm, decreased survival (males 52% vs. 82%, controls; females 54% vs. 76%, controls), decreased mean body weight and weight gain, primarily during the middle of the study (body weight -3% to -8% less than controls and gain at 53 weeks -12%, females and -16%, males); decreased mean body weight at termination in females (-8%) but not males; slightly decreased RBC count in males (-15%); decreased T4 in females (-28%, week 39); increased abs/rel liver weights (at wks 39 and 79, males +34%/+40% and +82%/+86%; females +57%/57% and +31%/31%), abs/rel thyroid weights (at wk 39, males >2-fold increase; females +30%) and abs/rel heart weights in females (+23%/+40%, wks 39 and 79); and increased incidence of hepatocellular hypertrophy in males and females (42% and 20% affected) and atrial thrombosis in males (16% vs. 2%, controls) and females (28%) were observed. **The systemic toxicity LOAEL is 640 ppm (123.3 mg/kg/day), based on hepatocellular hypertrophy in females. The NOAEL is 150 ppm (28.7 mg/kg/day). (In males, the systemic toxicity LOAEL is 3000 ppm or 467.6 mg/kg/day, based on decreased body weight/weight gain, increased thyroid and liver weights and hepatocellular hypertrophy. The NOAEL is 640 ppm or 98.6 mg/kg/day).**

Hepatocellular adenoma showed statistically significant, dose-related increases in both sexes at 3000 and 7000 ppm (from control to high dose, 9%, 17%, 17%, 42% and 57%, males and 0%, 0%, 8%, 24% and 56%, females; all animals on study) based on incident/statistical analysis conducted by HED (memo from L. Brunsman to N. McCarrol/L. Hansen, March 16, 2000).

Dosing was considered adequate in both sexes based on body weight/weight gain decreases and increased thyroid weights in males, liver effects in both sexes and atrial thrombosis in females at 3000 ppm, as well as additional effects at 7000 ppm.

This study is classified **Acceptable (§83-2b)** and satisfies the guideline requirement for a carcinogenicity study in the mouse.

Discussion of Tumor Data: The incidence of hepatocellular adenoma was increased (statistically significant, $p<0.01$) in both males and females at 3000 and 7000 ppm, the highest two dose levels tested. From control to high dose, the incidence was 9%, 17%, 17%, 42% and 57%, males and 0%, 0%, 8%, 24% and 56%, females.

Adequacy of the Dose Levels Tested: Dosing was considered adequate in this study based on decreased body weight/weight gain and increased thyroid weights in males, liver effects in both sexes and atrial thrombosis in females at 3000 ppm, as well as additional effects at 7000 ppm

1.1.4 Developmental/Reproductive Toxicity: Developmental and reproductive toxicity studies on thiophanate-methyl are summarized below in Table 2. Developmental toxicity was observed in the fetuses of rabbits exposed to 40 mg/kg/day thiophanate-methyl and included increased incidence of supernumerary ribs and decreased fetal weight. These findings occurred at a dose that also caused maternal toxicity based on decreases in body weight gain and food consumption. There were no abnormalities observed in the rat at gavage doses up to 1000 mg/kg/day or in the rat dietary developmental study as doses up to 163 mg/kg/day. Increased offspring sensitivity was not observed in the reproductive toxicity studies. In the 2-generation reproductive toxicity study, parental toxicity was observed at all doses tested ($\geq$13.7 mg/kg/day) based on mild hepatocellular hypertrophy and thyroid hypertrophy/hyperplasia, whereas offspring

toxicity was observed at ≥43.3 mg/kg/day as slightly reduced body weights of the F2b offspring during lactation. Although the offspring NOAEL and LOAEL (8 mg/kg/day and 32 mg/kg/day) were lower than the parental systemic NOAEL and LOAEL (≥32 mg/kg/day and >32 mg/kg/day) in the 3-generation reproductive toxicity study, liver and thyroid of parental animals were not evaluated and therefore the evidence for increased offspring susceptibility in that study is considered equivocal.

Table 2: Developmental and reproductive toxicity studies on thiophanate-methyl

Guideline	Study	Doses tested (mg/kg/day)	Results
870.3700a [83-3(a)]	Developmental toxicity study in Crl: COBS CD rats (gavage) MRID No. 00106090 Date: 1981 Unacceptable-guideline (upgradable with submission of dosing solution analyses, maternal clinical sign and food consumption data, and individual litter data)	0, 100, 300 or 1000 (gavage in 5% aq. gum arabic) tech., 97.2% a.i.	Maternal NOAEL = 300 mg/kg/day* Maternal LOAEL = 1000 mg/kg/day*, based on decreased body weight gain. Developmental NOAEL ≥1000 mg/kg/day* Developmental LOAEL >1000 mg/kg/day* * All endpoints tentative pending submission of additional information to upgrade study
870.3700a [83-3(a)]	Developmental toxicity study in Crl: COBS CD rats (diet) MRID No. 00146643 Date: 1985 Acceptable-guideline	0, 18, 85, or 163 (0, 250, 1200 or 2500 ppm in diet) tech., 95.3% a.i.	Maternal NOAEL = 18 mg/kg/day Maternal LOAEL = 85 mg/kg/day, based on decreased food consumption. Developmental NOAEL ≥163 mg/kg/day (HDT) Developmental LOAEL none established
870.3700b [83-3(b)]	Developmental Toxicity Study in New Zealand White Rabbits MRID No. 40028801, 41056701 Date: 1986 Unacceptable-nonguideline	0, 2, 6 or 20 (gavage in 1% aq. methyl cellulose) tech., 96.2% a.i.	Maternal NOAEL = 6 mg/kg/day Maternal LOAEL = 20 mg/kg/day, based on transiently decreased body weight gain, increased abortion/total litter loss Developmental NOAEL = ≥20 mg/kg/day Developmental LOAEL = none
870.3700b [83-3(b)]	Developmental Toxicity Study in New Zealand White Rabbits MRID No. 45051001 Date: 1997 Acceptable-guideline	0, 5, 10, 20 or 40 (gavage in 1% aq. methyl cellulose) tech., 97.28 a.i.	Maternal NOAEL = 10 mg/kg/day Maternal LOAEL = 20 mg/kg/day, based on decreased body weight gain and food consumption Developmental NOAEL = 20 mg/kg/day Developmental LOAEL = 40 mg/kg/day, based on increased supernumerary ribs and decreased fetal weight.

Table 2: Developmental and reproductive toxicity studies on thiophanate-methyl			
Guideline	**Study**	**Doses tested (mg/kg/day)**	**Results**
870.3800 [83-4]	Two-Generation Reproductive toxicity Study in Crl:CD(SD)BR Rats MRID Nos. 42799101 to -05; 43624401 Date: 1993 (addendum 1995) Acceptable-guideline	♂ 0, 13.7, 43.3 or 138.9; ♀ 0, 15.5, 54.0 or 172.0 (in diet) tech., 95.9% a.i.	Parental systemic NOAEL <13.7 mg/kg/day Parental systemic LOAEL = 13.7 mg/kg/day, based on hepatocellular hypertrophy and thyroid hypertrophy/hyperplasia. At ≥43.3 mg/kg/day, slightly decreased body weight gains in males and at 138.9 mg/kg/day, increased liver and thyroid weights (both sexes). Reproductive NOAEL ≥ 138.9 mg/kg/day (HDT) Reproductive LOAEL > 138.9 mg/kg/day Offspring NOAEL = 13.7 mg/kg/day Offspring LOAEL = 43.3 mg/kg/day, based on slightly reduced body weights of the F2b offspring during lactation.
870.3800 [83-4]	Three-Generation Reproductive Toxicity Study in CD Rats MRID No. 00117870 Date: 1972 Unacceptable-guideline (upgradable with submission of test material purity)	0, 2, 8 or 32 (estimated from ppm in diet) purity a.i. not stated	Parental systemic/reproductive NOAEL ≥32 mg/kg/day Parental systemic/reproductive LOAEL >32 mg/kg/day Offspring NOAEL = 8 mg/kg/day Offspring LOAEL = 32 mg/kg/day, based on slightly decreased mean litter weights.

(1) Developmental Toxicity:

Rat - Two developmental toxicity studies were available in this species; in one study, thiophanate-methyl was administered via gavage and in the other via dietary administration.

In the **gavage study,** (MRID 00106090), 25 presumed pregnant Charles River COBS® CD® rats per group were administered Thiophanate-methyl (97.2% a.i.; Lot No. TM-123) daily by **gavage** at doses of 0, 100, 300 or 1000 mg/kg/day on gestation days (GD) 6-19, inclusive. On GD 20, dams were sacrificed and subjected to gross necropsy. At necropsy, the fetuses were weighed, sexed and examined for external malformations and variations, including the palate and eyes. Approximately one-half of the fetuses were placed in Bouin's fixative for subsequent soft tissue examination. The remaining one-half of the fetuses were fixed in alcohol, macerated in potassium hydroxide and stained with Alizarin Red S for subsequent skeletal examination.

At 1000 mg/kg/day, a statistically significant reduction in body weight gain during the first 3 days of treatment (-100% of controls from GD 6-9, $p<0.05$) was observed. Reduced body weight gain (not statistically significant) during the dosing interval of GD 9-12 (-17%), and for the entire dosing period (-10%; measured from GD 6-20) was observed. Mean absolute body weights were not significantly affected by treatment. At 100 and 300 mg/kg/day, body weight gain was statistically

significantly reduced ($p<0.05$) during the first three days of treatment as compared to controls (-56% of controls for both the 100 and 300 mg/kg/day groups); however, body weight gain in these groups was comparable to controls for the remainder of dosing and over the entire dosing period (measured between GD 6-20). No treatment-related gross pathological changes were noted. Individual or summary data of maternal antemortem/daily observations were not included: the study author stated in the text that no treatment-related clinical signs of toxicity were noted. **The maternal toxicity LOAEL is 1000 mg/kg/day based on reduced body weight gain. The maternal toxicity NOAEL is 300 mg/kg/day.** This LOAEL/NOAEL is contingent upon individual/summary data of clinical signs not revealing any significant treatment-related effects at lower doses, and upon test substance analyses verifying that test material stability, homogeneity, and concentration of the dosing medium are acceptable.

No developmental effects were reported in the data provided by the study author in the summary tables at any dose tested; however, incidence rates or calculation of overall litter incidences for malformations/variations could not be conducted because individual fetal data were not provided. **Therefore, a tentative developmental toxicity LOAEL is >1000 mg/kg/day and NOAEL is ≥1000 mg/kg/day.**

This study is classified as **Unacceptable (§83-3(a))** (**upgradable**) and does not satisfy the Subdivision F requirements for a developmental toxicity study in rats because analyses for test material stability, homogeneity, and concentration in dosing medium were not provided, individual or summary data of maternal antemortem/daily observations were not available, food consumption data were not provided and individual fetal examination data for external, visceral, and skeletal variations were not provided. This study may be **upgradable** if the missing data are supplied. The reduced number of litters in most groups (18, 19, 17 and 16 for the 0, 100, 300 and 1000 mg/kg/day groups, respectively; guideline minimum is 20 litters/group) and the lack of adjustment for dose concentrations with increases in body weights are not considered to have significantly compromised the study conclusions. The limit dose was tested and the pilot study testing doses of up to 5000 mg/kg/day resulted only in moderate maternal body weight decreases.

In 1985, HED reevaluated another developmental toxicity study in rats by the dietary route of administration. This study was requested since the results of the 2-generation reproduction study (MRID No.:42899101 to -05 and 43624401) indicated that dietary administration is the preferred route of exposure for assessing thiophanate methyl for teratogenic potential. (Memorandum from R. Gardner to P. Hundemann dated May 21, 1985, HED Document No.: 004459).

In the **dietary** administration study, (MRID 00146643), 25 presumed pregnant Crl:COBS® CD® (SD) BR strain rats per group were fed Thiophanate-methyl (95.3% a.i.) at dietary concentrations of 0, 250, 1200 or 2500 ppm (equivalent to 0, 18, 85 or 163 mg/kg/day) on gestation days (GD) 6 through 19, inclusive. On GD 20, dams were sacrificed and subjected to gross necropsy. At necropsy, the gravid uteri and individual fetuses from each dam were weighed, and the numbers of corpora lutea, implantation sites, live and dead fetuses, and embryonic deaths were noted. Crown-to-rump length was measured for all fetuses. All fetuses were sexed and examined for external malformations and variations.

Maternal Toxicity: Decreased food consumption and associated body weight effects were noted at 1200 and 2500 ppm levels. The reduced food consumption was significant during days 6-12 for

the1200 ppm group (13-18% reduction) and days 6-15 for 2500 ppm group (17 to 40% reduction) when food consumption was decreased as much as 40% just after dosing began (days 6-9 for 2500 ppm group). The decreased food consumption in the 1200 and 2500 ppm groups was accompanied by decreases in net body weight (2% and 5.6% decreases relative to controls without gravid uterus, respectively), body weight gain (25% and 36% decreases relative to controls, respectively), and uterine weight (3.4% and 11.3%, respectively) but the effects were reversible. It is possible that the decreased food consumption was due to decreased palatability of the test diets. Alopecia was the most frequently reported clinical sign that was observed in 3-6 animals in all groups and was noted prior to study initiation. The frequency and number of animals affected by alopecia was slightly higher in the high dose group. There were no significant differences reported between groups for number of corpora lutea, implantations, resorptions (early and late), or live fetuses per dam. **The maternal toxicity NOAEL is 250 ppm (18 mg/kg/day) and the maternal toxicity LOAEL is 1200 ppm (85 mg/kg/day) based on significantly reduced food consumption.**

Developmental Toxicity: There were no treatment-related effects on fetal weight, crown to rump length, external variations or fetal malformations. The most frequently reported soft tissue observation was undeveloped renal papillae and/or distended ureters that occurred in 10, 4.3, 25 and 13% of the litters, of the 0, 250, 1200 and 2500 ppm groups, respectively. These observations were not considered treatment-related because they were not dose-related. The most frequently reported skeletal variations were unossified sternebrae (#5 and #6) that occurred in 55, 52.2, 66.7 and 65.4% of the litters (12.4, 17.4, 17.3 and 15% of fetuses), respectively, and 14th rudimentary ribs that occurred in 45, 47.8, 41.7 and 39.1% of the litters (5.4, 5.4, 4.4 and 3.7% of fetuses), respectively. However, the incidence of unossified sternebrae in fetuses and litters was not statistically significant, and was within the reported historical control range. **The developmental toxicity NOAEL is 2500 ppm (163 mg/kg/day) (highest dose tested) based on an absence of treatment-related effects in the fetuses.**

This study is classified as **Acceptable-Guideline**, and does satisfy the §83-3 (a) Subdivision F requirements for a developmental toxicity study in rats.

The gavage study in which no maternal nor developmental toxicity were seen at the Limit Dose was originally classified as Unacceptable but upgradable pending receipt of additional information (e.g., analysis of the dosing solution, individual animal data) requested by HED. In light of the results of the gavage study, the Agency requested a dietary administration study to evaluate the teratogenic potential of thiophanate methyl when administered in the diet rather than gavage.

The dietary study also showed no developmental toxicity at the highest dose tested (2500 ppm or 163 mg/kg/day). The results of the two studies are consistent with each other and the two studies together establishing NOAEL for maternal toxicity based on reduced food consumption. In addition, a pilot gavage study at doses up to 5000 mg/kg/day only moderate maternal body weight decrements were seen. The TOX SAC recommended (refer to TOX SAC report from Joycelyn E. Stewart dated March 23, 2000) that the dietary developmental toxicity study in rats be classified as ACCEPTABLE-guideline and that the study satisfies the requirement for a Subdivision F guideline study for developmental tonicity in the rat. Consequently, it is not surprising that only minimal toxicity was seen via the dietary study. In summary, the results of these two studies together indicate that thiophanate-methyl is not a developmental toxicant in rats.

Rabbit - Two developmental toxicity studies were available in this species. In a study conducted in 1986 in a testing facility in England and the other at a testing facility in the U.S.A

First Study (1986).

Executive Summary: The following Executive Summary has been revised by J. Doherty as per the outcome of this HIARC meeting.

In a developmental toxicity study (MRID 40022801, 41056701) 15 pregnant (artificially inseminated) New Zealand white rabbits/dose group were administered thiophanate-methyl (tech., 96.2% a.i.) by gavage at doses of 0, 2, 6 or 20 mg/kg/day from days 6 through 19 of gestation. Test material was administered in 1% w/v aqueous methyl cellulose.

Maternal Toxicity: At 20 mg/kg/day, decreased mean body weight (transient, maximum -8.6% below controls at day 10 due to weight loss during days 6-10 of gestation), decreased food consumption (transient, -38% between days 6-12 of gestation), decreased fecal output and increased incidence of abortion/total litter loss (2 abortions/1 total litter loss vs. 0 abortions/1 total litter loss, controls) were observed. No clinical signs or gross findings were observed. **The maternal toxicity LOAEL is 20 mg/kg/day, based on increased incidence of abortions and decreases in body weight and food consumption. The NOAEL is 6 mg/kg/day.**

Developmental Toxicity: An issue of a possible increase in "asymmetric pelvis" was indicated by there being an apparent increased incidence (not statistically significant, fetal incidence 7.4% vs. 3.4%, controls; litter incidence 42% vs. 25%, controls) at 6 mg/kg/day. At 20 mg/kg/day, asymmetric pelvis at fetal incidence of 9.8% exceeded incidence reported for available historical control data; litter incidence was 56% (historical litter incidence not available); neither was statistically significant. This was considered an equivocal finding and a second study in rabbits was conducted and no pelvic abnormalities were noted. There was also an increased fetal and litter incidence of thickened ribs at costal cartilage (13.7% vs. 1.1%, controls) was considered of uncertain toxicological significance at 20 mg/kg/day. No effects on pup weight were observed. The developmental toxicity NOAEL is greater than 20 mg/kg/day for this study because the asymmetric pelvis seen in this study was not seen at all even at higher doses in the 1997 rabbit study. The developmental NOAEL of 20 mg/kg/day and the LOAEL of 40 mg/kg/day will be based on the 1997 rabbit study. The LOAEL is based on supernumerary ribs and decreases in fetal weight.

In light of the equivocal findings in the 1986 study, HED recommended a new study to confirm or otherwise resolve the significance of these finding (refer to memo from R. Gardner to P. Hundemann dated April 15, 1987, HED Document No.: 005840).

The 1997 study is considered to be more appropriate to satisfy the developmental toxicity study in rabbits and the 1986 study is considered to have technical problems such as: 1) current standards as used in the 1997 study require that dosing be continued to day 28 of gestation, in the 1986 study dosing was on days 18-19 only; 2) there was the possibility of poor animal health as indicated by intercurrent infection and some animals were sacrificed *in extremis* (two controls and one in each dose group); 3) in the 1986 study at gestation day 29, Caesarian sectioning revealed animals with intrauterine infection, there were also abortions in the 1986 study since none were seen in the 1997 study; 4) fewer animals (only 9 to 12) were pregnant and had live fetuses available for examination in the 1986 study, the 1997 study had more pregnant does (16 to 19) and live fetuses; and 5) there were data reporting problems in correlating the effects reported to individual fetuses. Because of

these reasons, the HIARC determined that this study should be classified as unacceptable/nonguideline.

Second Study (1997).

In the 1997 study, (MRID 45051001) thiophanate-methyl (97.28% purity) was administered to groups of 20 New Zealand White Rabbits by gavage in a 1% aqueous methyl cellulose vehicle (at a rate of 10 mL/kg) at dose levels of 0, 5, 10, 20 or 40 mg/kg/day on gestation days 6 to 28. The rabbits were sacrificed on day 29 and the does were subjected to uterine examination and the pups subjected to external, visceral and skeletal examination.

At 20 mg/kg/day there was **decreased body weight *gain*** (56%, < 0.05) for the interval days 12-15 and body weight gain was decreased 13% for the entire dosing period. At 40 mg/kg/day, body weight gain was decreased and there was actual body weight loss for the interval days 6-9 (.i.e.. the controls gained 80±40 g while the 40 mg/kg/day dose group actually lost 110±100 g). Final (day 29) body weight of the does in the high dose group was 6% less than the control. **Decreased food consumption** accompanied the decease in body weight with there being 13 to 20% decrease in the 20 mg/kg/day dose group and 24 to 70% decreased in the high dose group. The high dose group also had more does with scant or no feces. There were no abortions. **The LOAEL for maternal toxicity is 20 mg/kg/day based on body weight and food consumption decreases. The NOAEL is 10 mg/kg/day.**

At 40 mg/kg/day, there were statistically significant ($p < 0.01$) *increases* in the mean number of ossification sites in the thoracic vertebrae (+3.12%) and ribs-pairs (+3.21%) as well as a *decrease* in lumbar vertebrae (-6%) and the differences were in excess of or less than the historical control range respectively. These conditions were collectively referred to as an *increase* in "**supernumerary ribs**" by the study author and were described as a reversible condition. There were also decreases (not statistically significant) in fetal weight (-9.6% for males and -6.6% for females). **The LOAEL is 40 mg/kg/day based on supernumerary ribs and decrease in fetal weight. The NOAEL is 20 mg/kg/day.**

Classification: This study is classified as **Acceptable - Guideline** and satisfies the requirement for a series 83-3 developmental toxicity study in rabbits.

(2) Reproductive Toxicity:

Rat - Topsin-M (95.9% a.i.) was tested in a two-generation reproduction study with male and female Sprague-Dawley Crl:CD(SD)BR rats (MRIDs 42799101 to -05, 43624401). The rats were administered the test material in the diet at concentrations of 0, 200, 630, or 2000 ppm (calculated to be 0, 13.7, 43.3 or 138.9 mg/kg/day for males and 0, 15.5, 54.0 or 172.0 mg/kg/day for females). Twenty-five animals/sex/dose/generation were selected for testing. The P generation animals were given test or control diet for 14 weeks (98 days) then mated to produce the F_1 animals. Approximately 14 weeks after weaning of all F_1 offspring, selected F_1 animals were mated within the same dose group for a maximum of 21 days (sibling matings were avoided) to produce the F_{2a} generation. After weaning of the F_{2a} pups, F_1 animals were maintained for 6 weeks and mated again to the same partner to produce the F_{2b} offspring. The second mating of the F_1 animals was performed due to a high, unexplained death rate in the F_{2a} treated and control pups during lactation. All animals were exposed to test material, either in the diet or during lactation, until sacrifice.

No clinical signs of toxicity or mortalities in the parental animals of either generation were attributable to treatment. There were no significant differences in body weights of the P generation high-dose males and the F_1 mid- and high dose males during the pre-mating periods when compared to controls, however, there was a slight dose-related reduction in body weights throughout the study. The premating period (days 1-43) body weight gains in the 630 and 2000 ppm F_{1b} males were less than controls: 56 and 55% of the control value, respectively. These were considered to be borderline significant because the changes were in the range of 5% of the total bodyweight. In females, although there were some decreases in bodyweight and bodyweight gain during gestation, these were not consistent across generations and/or litters and were thus not biologically significant. High-dose P generation males and females and high-dose F_1 males had significantly (p 0.05) increased liver and thyroid weights and high-dose F_1 females had increased thyroid weights when compared to controls. Increased organ weights correlated with statistically significant increases in hepatocellular hypertrophy and thyroid follicular cell hyperplasia/hypertrophy in the high dose group. Generally, minimal to slight hepatocellular hypertrophy and thyroid follicular cell hypertrophy and hyperplasia were observed in both the low and mid-dose P generation males. These effects were observed in the F_1 generation but appeared in fewer animals and were less severe. In females, these effects were considerably less. **Therefore, the NOAEL for systemic toxicity is <200 ppm (13.7 mg/kg/day) based on hepatocellular hypertrophy and thyroid follicular cell hypertrophy/hyperplasia at all dose levels and decreased body weight gains in males and increased liver and thyroid weights in both sexes at the highest dose level. This LOAEL is considered to be a borderline NOAEL/LOAEL because the effects on the thyroid and liver at 200 ppm were minimal and they were less in the succeeding generation**.

No treatment-related effects were noted on the reproductive performance indices of either generation. Mean litter sizes, survival indices, and sex ratios were not different between treated and control groups for the F_1 and F_{2b} offspring. Due to a high rate of death in both the treated and control F_{2a} pups, a second mating of the F_1 animals was made to produce the F_{2b} offspring. Deaths of the F_{2a} pups did not appear to be treatment-related as controls were equally affected and the result was not repeated after the second mating. No statistically significant differences occurred in mean pup weights from any treated group at any time during lactation of the F_1 or F_{2a} pups. However, F_{2b} pups gained less weight than controls with day 21 body weights of 630 and 2000 ppm group males and females being 88% of the control value. When mean pup weights for the F_{2b} litters were analyzed by covariance analysis (ANCOVA) to account for the number of pups per litter, significantly lower weights as compared to control were seen for the 630 ppm males and females on day 1 (p 0.01), 2000 ppm males on day 21 (p 0.05), and 630 and 2000 ppm females on day 21 (p 0.05). Decreased F_{2b} pup weights were not coincident with reduced dam weights since high-dose dams actually gained slightly more than controls during lactation. **Therefore, the LOAEL for offspring toxicity is 630 ppm (43.3 mg/kg/day) based on reduced body weights of the F_{2b} pups during lactation. The corresponding NOAEL is 200 ppm (13.7 mg/kg/day). This LOAEL is also considered to be borderline because the decrease in pup weights was minimal. The reproductive toxicity LOAEL is >2000 ppm (138.9 mg/kg/day) and the NOAEL is ≥2000 ppm.**

This study is classified as Acceptable and satisfies the guideline requirements for a multigeneration reproduction feeding study (83-4).

Rat - In a 3-generation reproductive toxicity study (MRID 00117870), 10 male and 20 female CD rats/dose group were administered thiophanate-methyl (purity not stated) at dietary concentrations of 0, 40, 160 or 640 ppm (equivalent to approximately 0, 2, 8 or 32 mg/kg/day, as estimated using ppm in diet and a standard factor of 0.05 to convert ppm to mg/kg/day in rats). Administration of

treated diets to weanling F0, F1b and F2b parental animals was initiated 60 days prior to mating. Each male was cohabited with 2 females. Two matings were conducted for each of the 3 generations. Control and high dose F3b offspring were also examined microscopically for soft tissue and skeletal abnormalities (10/sex each) and weights of many major organs were also measured.

Parental/reproductive toxicity: There were no treatment-related effects on mortality, clinical signs, body weight, food consumption or gross findings in the parental animals. No effects on reproductive parameters were observed. **The LOAEL for parental systemic/reproductive toxicity is >640 ppm (32 mg/kg/day). The NOAEL is ≥640 ppm.**

Offspring toxicity: At 640 ppm, slightly decreased mean litter weights were observed in both mating of all 3 generations except for the F3a litters (-3.7% to -15.4% less than controls; not statistically significant). This was attributed in part to slightly lower litter sizes as well as lower individual pup weights. These decreases tended to continue throughout lactation in most groups (at lactation day 21, -5.5% to -17% less than controls; F1b and F3a weights not decreased). There were no treatment-related effects on viability indices or gross findings. F3b animals examined microscopically showed no treatment-related abnormalities and no organ weight changes. **The LOAEL for offspring toxicity is 640 ppm (32 mg/kg/day), based on slight but consistently observed decreases in mean litter weights (5 of 6 matings). The NOAEL is 160 ppm (8 mg/kg/day).**

This study is classified **Unacceptable (§83-4)-Upgradable**. Although the study appeared to have been appropriately conducted, purity of the test material was not given in the report. However, an acceptable rat multigeneration reproduction study was previously submitted (MRID 42799101 through -05 and 43624401; reviewed in HED Doc. No. 011748) which satisfies the guideline requirement for 83-4. No further information is therefore needed at this time to satisfy this requirement.

1.1.5 Neurotoxicity: No acute or subchronic rodent neurotoxicity screening studies (§81-8 and §82-7) were submitted for thiophanate-methyl. The HIARC (meeting of 4/8/99) determined that these studies should be submitted based on (1) potential clinical signs of neurotoxicity in the chronic dog study (transient tremors) and (2) existence of a common metabolite, MBC, with benomyl. In an earlier HIARC meeting (memorandum from J. Rowland to B. Madden, 12/3/97; HED Doc. No. 012418), it was determined that benomyl, which has a metabolite in common with thiophanate-methyl (MBC), showed potential signs of neurotoxicity in the acute and subchronic rat neurotoxicity screening studies. In addition, in the rat developmental toxicity studies, both MBC (MRID No. 40438001) and benomyl (MRIDs 00148393, 00119017) caused developmental neurotoxic effects. A developmental neurotoxicity study (§83-6) was therefore requested for benomyl. A developmental neurotoxicity study is also required for thiophanate-methyl because of these concerns and because of concern for potential effects on the development of the nervous system development if thiophanate-methyl has antithyroid activity.

1.1.6 Genotoxicity: Although the acceptable submitted genotoxicity studies (*in vitro* CHO cytogenetic and rat liver unscheduled DNA synthesis assays) were negative, two published reports (mouse bone marrow micronucleus and BALB/c 3T3 cell transformation assays) demonstrated that thiophanate-methyl is aneugenic. Weak equivocal positive results were observed in a published Ames assay. The available studies are summarized below in Table 3. The CARC determined that additional genotoxicity testing should be provided to adequately assess direct mutagenicity of thiophanate-methyl: (1) a *Salmonella typhimurium* mammalian microsome gene mutation assay

(pre-incubation modification) to resolve the equivocal results from the literature; (2) a mouse lymphoma L5178Y mammalian cell forward gene mutation assay, including colony sizing; (3) an *in vivo* mouse micronucleus assay should be performed and the Agency prefers that this assay include immunofluorescent antikinetochore-specific antibody staining. Finally, (4) the 2-aminobenzimidazole metabolite of thiophanate-methyl should be tested at minimum in the *S. typhimurium* mammalian microsome gene mutation assay because of the structural alert for mutagenesis (i.e, the NH_2 group attached to the imidazole ring).

Table 3: Genotoxicity studies on thiophanate-methyl

Guideline	Study	Doses	Results
870.5100 [84-2(a)]	Preincubation *Salmonella typhimurium* Mammalian Microsome Gene Mutation Assay Published report (Zeiger *et al.*, 1992; not submitted to the Agency) Accepted for regulatory purposes	0 to 10,000 g/plate with or without rat or hamster liver S9. Tech., 95.1% a.i.	Weak equivocal response: 2-fold increases in revertant colonies of strains TA98 and TA100 at ≥3333.0 g/plate (precipitating concentration) with S9 and negative results in second assay. Negative response without S9.
870.5375 [84-2(b)]	*In Vitro* Mammalian Cell Cytogenetic Assay in Chinese Hamster Ovary (CHO Cells MRID No. 40980101 Date: 1988 Acceptable-guideline	0 to 400 g/ml culture medium without rat liver S9 and 0 to 1000 g/ml with S9 Tech., 95% a.i.	Negative for structural chromosomal aberrations. Mitotic delay increased at 100 g/ml without S9 and 335 g/ml with S9. Cytotoxicity/compound insolubility observed at 400 g/ml without S9 and 750 g/ml with S9.
870.5385 [84-2(b)]	*In Vivo* Mouse Bone Marrow Micronucleus Assay Published report (Barale, 1993; not submitted to the Agency) Accepted for regulatory purposes	1 mg/kg body weight, single gavage dose Tech., 95% a.i.	Borderline significant increase in polyploidy and hyperploidy. No increase in structural chromosomal aberrations.
870.5550 [84-4]	*In Vitro* Unscheduled DNA Synthesis Assay in Primary Rat Hepatocytes MRID No. 40095503 Date: 1981 Acceptable-guideline	0 to 1000 g/ml culture medium tech., 99.8% a.i.	Negative for UDS induction at all doses tested. Cytotoxic at 1000 g/ml.
No guideline # [84-4]	*In Vitro* Cell Transformation Assay in BALB/c 3T3 Cells Published report (Perocco *et al.*, 1997; not submitted to the Agency) Accepted for regulatory purposes	0 to 200 g/ml culture medium with rat liver S9; 0 to 25 g/ml without S9 Tech., 99.5% a.i.	Significant and reproducible increase in morphologically transformed foci at 25 g/ml without S9 and ≥20 g/ml with S9. Cytotoxicity observed at ≥25 g/ml (pronounced at ≥50 g/ml) without S9; only weak cytotoxicity with S9 (most pronounced at 100-200 g/ml).

1.1.7. Metabolism: Results of a metabolism study in the rat are summarized below in Table 4. There was no significant retention of thiophanate-methyl or its metabolites in tissues and most of the administered dose was excreted within 24 hrs post-dosing. The extent of metabolism of parent

compound and amount of radioactivity excreted in urine vs. feces was less in the single oral high dose and repeated low dose groups compared to the single low dose group. The metabolite MBC is also a metabolite of the fungicide benomyl.

Table 4: Metabolism of Thiophanate-Methyl

Guideline	Study	Doses	Results
870.7485 [85-1]	Metabolism and pharmacokinetics study in the F344 rat MRID Nos. 42474802 and 42601601 Date: 1992 Acceptable-guideline	low oral radiolabelled 14 mg/kg; repeated oral unlabeled 14 mg/kg for 14 days, followed by single radiolabelled; high oral radiolabelled 170 mg/kg Tech., 97.3%-98.5% radiochemical purity ^{14}C-thiophanate-methyl	Thiophanate-methyl was rapidly absorbed, metabolized and excreted at all dose levels (>90% within 24 hrs). Radioactivity did not accumulate in tissues (highest concentrations were in thyroid, 0.04-2.49 g/g; liver, 0.17-2.15 g/g; kidney 0.04-0.51 g/g). Plasma half life for low, high and repeated doses was 2.8, 2.2 and 7.8 hrs, males and 2.5, 1.6 and 4.0 hrs, females. T_{max} was achieved at 1-2, 2-3 and 4-7 hrs at single low, repeated low and single high doses, respectively. The primary route of excretion was urinary following a single low oral dose (70-72% of administered radioactivity) but was fecal after repeated low (48-49%) or single high (67-70%) doses. Excretion in CO_2 was negligible. Metabolite profiles were qualitatively similar for all groups. Twelve identified and 4 unknown urinary metabolites were identified, including methyl 2-benzimidazolylcarbamate (MBC, 0.2 to 2.2% of recovered radioactivity) and other sulfate-conjugated and/or hydroxylated derivatives of the parent compound. The major urinary metabolite was 5-hydroxy(2-methoxycarbonylamino) benzimidazolyl sulfate (14-42%). Seven identified and 2 unknown fecal metabolites were identified; the major fecal metabolite was dimethyl[1,2-(4-hydroxyphenylene)]bis (iminocarbonothioyl)bis (carbamate) (3.5-11%). MBC was also identified in feces (0.5-2.7%). After a single low dose the parent compound was almost completely metabolized (1% of dose excreted), but it was the major excreted compound in feces of the repeated low dose (21-24%) and single high dose (52-56%) groups. No significant differences in metabolism were reported between males and females.

1.1.8 Mechanistic Studies: In order to characterize the mechanism of thyroid tumorigenesis, a series of short-term studies were undertaken to determine whether thiophanate-methyl had antithyroid activity. The results of these assays are summarized below in Table 5. These studies demonstrated that thiophanate-methyl caused liver and thyroid enlargement, increased circulating TSH and decreased T3/T4 after 2 to 8 days' treatment with thiophanate-methyl at 6000 ppm (equivalent to the HDT in the rat chronic toxicity/carcinogenicity study). Some liver microsomal enzymes, including UDP-glucuronosyltransferase, were increased. The effects on liver and thyroid weight were reversible, but reversibility of the alterations in circulating hormone levels and on microscopic effects were not evaluated. Supplementation of treated animals with T4 prevented thyroid enlargement and increased TSH but did not prevent liver enlargement. Thiophanate-methyl also appeared to have a mild inhibitory effect on microsomal thyroid peroxidase. These data were reviewed by the HED CARC. Although it was determined that the available evidence is consistent with disruption of thyroid-pituitary homeostasis by thiophanate-methyl, additional data were considered necessary to adequately support this mechanism. The current Agency policy on rat

thyroid tumors (US EPA, 1998) requires demonstration of the reversibility of the thyroid hormonal alterations and microscopic changes after withdrawal of treatment; these data demonstrated only reversibility of thyroid weight. In addition, there were insufficient genotoxicity data for evaluation of direct mutagenicity of thiophanate-methyl.

Table 5: Special thyroid and liver mechanistic studies, supplement to chronic feeding/oncogenicity study in rats (MRID 42896601b; 1996-Acceptable/Non-guideline)

Guideline	Purpose of study	Doses	Results
None	(1) Effect of short-term dietary administration of TM on liver and thyroid weights; circulating T3/T4 and TSH and serum cholesterol in male F344 rats	0 or 6000 ppm for 2 or 8 days Tech., 96.55% a.i. (all experiments in this study) Positive control groups: 500 ppm phenobarbital (liver enlargement) and 1000 ppm propylthiourea (PTU; antithyroid activity)	TM caused liver and thyroid enlargement; increased serum cholesterol and TSH; decreased T3 and T4 (decreases marginal at day 8). PB caused liver enlargement and increased T3, T4, TSH and cholesterol at day 8. PTU caused thyroid and liver enlargement; increased TSH and cholesterol; decreased T3 and T4 (slight).
	(2) Reversibility of thyroid enlargement following termination of short-term dietary administration of TM in female F344 rats	0 or 6000 ppm for 8 days; half sacrificed on day 8 and half given basal diet for 8 additional days Positive (liver)/negative (thyroid) control group: 500 ppm phenobarbital	Withdrawal of TM after 8 days' treatment caused reversal of the thyroid enlargement. Treatment with PB for 8 days' and subsequent withdrawal and recovery had no significant effect on thyroid weight.
	(3) Effect of T4 supplementation on thyroid and liver weights, TSH and serum cholesterol during short-term dietary administration of TM in male F344 rats	0 or 6000 ppm for 8 days; half of animals also received daily injections of 30 /kg L-thyroxine	Supplementation with T4 prevented thyroid enlargement and increased TSH but not liver enlargement or increased serum cholesterol.
	(4) Effect of TM on hepatic microsomal enzyme activities and protein concentration following short-term administration of TM to male F344 rats (livers collected from animals of study 1)	0 or 6000 ppm for 8 days Positive control: 500 ppm PB for 8 days	TM caused an increase in cytochromes p-450 and b5, and a pronounced increase in UDP-glucuronosyltransferase. Microsomal protein was also increased. PB caused an increase in cytochromes p-450 and b5, NADPH-cytochrome c reductase, UDP-glucuronosyltransferase and microsomal protein.
	(5) Effect of TM on porcine microsomal thyroid peroxidase activity	10^{-3} to 10^{-4}M, Guaiacol method Positive control: 10^{-4} to 10^{-6} PTU	The ED_{50} (effective dose to achieve 50% inhibition of thyroid peroxidase) for TM was 6×10^{-4}M and no inhibition was reported at 8×10^{-5} M (about 30-fold greater than PTU). The ED_{50} for PTU was 2×10^{-5}M and no inhibition was reported at 4×10^{-7}M.

Table 5: Special thyroid and liver mechanistic studies, supplement to chronic feeding/oncogenicity study in rats (MRID 42896601b; 1996-Acceptable/Non-guideline)			
Guideline	**Purpose of study**	**Doses**	**Results**
	(6) Effect of TM on hepatocyte proliferation as measured by PCNA immunohistochemical staining following treatment with TM in male F344 rats and ICR mice	0 or 6000 ppm for 2 or 8 days Positive control: 500 ppm phenobarbital	In mice, TM caused a sustained increase in PCNA staining and liver enlargement after 2 and 8 days' treatment. In rats, PCNA staining was increased at day 2 but not day 8; liver weights were increased at both times. In mice, PB caused increased PCNA staining at days 2 and 8 but less pronounced at day 8 than day 2. In rats, PCNA staining was increased at day 2 but not day 8. Liver weights were increased at both times.

1.1.9 Structure-Activity Relationship (SAR) Considerations: Thiophanate-methyl shares a common metabolite, MBC, with the fungicide benomyl. Both benomyl (MRID 00096514) and MBC (Accession Nos. 256028, 256029, 2560302) cause liver tumors in mice, but do not affect the thyroid in rats (MRID 00088333; study conducted on MBC). Benomyl and MBC are also aneugenic (but not clastogenic) as demonstrated by positive results in mouse bone marrow micronucleus (MRID 41051510; Barale *et al.*, 1993) and Chinese Hamster ovary cell chromosomal aberration (MRID 41184601) assays. Benomyl and MBC also increased the frequency of kinetochore-positive micronuclei in mice (MRIDs 42911601, -02). Benomyl appears to be only weakly mutagenic in bacteria and cultured mammalian cells and conflicting results have been obtained in *in vitro* chromosomal structure assays (cell cycle delays but no effect on chromosomal structure; MRID 41051523). In developmental toxicity studies, benomyl caused ocular and CNS malformations in rats (MRID 00148393) but developmental toxicity was not reported in the rabbit (MRID 43788301). MBC caused axial skeletal abnormalities, increased early resorptions, decreased live litters and litter weights and eye and CNS effects in rats (MRID 40438001). A safety factor of 10 was retained and a developmental neurotoxicity study required for benomyl based on these findings (memorandum from J. Rowland to B. Madden, dated 12/3/97; HED Doc. No. 012418).

Benomyl is converted more rapidly to MBC than thiophanate-methyl (Selling *et al.*, 1970). Although the relative amount of MBC identified in urine and feces in the rat metabolism study on thiophanate-methyl is low (0.2-2.7% of administered radioactivity; see Table 4, above), because of this common metabolite, HED has concerns for potential developmental toxicity and neurotoxicity from exposure to thiophanate-methyl. The FQPA Safety Factor Committee's decision to retain this factor for thiophanate-methyl and require a developmental neurotoxicity study are based in part on this SAR (see Section 3.3, below, on FQPA Considerations).

A second structure-activity consideration is the presence of the thiourea group in thiophanate-methyl and some of its metabolites. This moiety is also observed in some thyroid carcinogens of the thionamide class such as propylthiouracil. Neither benomyl nor MBC have metabolites containing this group. The toxicity of thiophanate-methyl may therefore be caused by more than one mechanism, e.g., effects caused by MBC, the thiourea moiety or others.

A closely related chemical, thiophanate-ethyl, did not cause thyroid or liver tumors in Sprague-Dawley rats up to 1000 ppm (MRID 00032673) or C57BL/6 mice up to 2000 ppm (MRID 00081605), but may not have been tested at high enough dose levels to induce these tumors, or there

may have been differences in responses in the strains used. However, it did induce thyroid hypertrophy and liver enlargement in the rat study. The only effect reported in the mouse carcinogenicity study was decreased spermatogenesis at 2000 ppm.

1.2 Acute Toxicity

Thiophanate-methyl possesses a low order acute toxicity by oral, dermal and inhalation routes of exposure (categories III/IV). It is only slightly irritating to the skin and is not an ocular irritant (both category IV), but is a dermal sensitizer. Studies are summarized below in Table 6:

Table 6: Acute Toxicity Profile of Thiophanate-Methyl (tech. a.i.)

Guideline No.	Study Type	MRID #	Results	Toxicity Category
870.1100 [81-1]	Acute Oral, Rat	41644301	LD_{50} >5000 mg/kg, both sexes	IV
870.1200 [81-2]	Acute Dermal, Rabbit	41644302	LD_{50} >2000 mg/kg, both sexes	III
870.1300 [81-3]	Acute Inhalation, Rat	41482804	LC_{50} = 1.7 mg/L, males 1.9 mg/L, females	III
870.2400 [81-4]	Primary Eye Irritation, Rabbit	40095501	Slight ocular irritant	IV
870.2500 [81-5]	Primary Skin Irritation, Rabbit	40095502	Not a dermal irritant	IV
870.2600 [81-6]	Dermal Sensitization, Guinea Pig	41482805	Is a dermal sensitizer	
870.6200 [81-8]	Acute Neurotoxicity, Rat	N/A		

N/A No data available

REFERENCES (PUBLICATIONS)

Barale, R., Scapoli, C., Meli, C., Casini, D., Minunni, M., Marrazzini, A., Loprieno, N. and Barrai, I. (1993) Cytogenetic effects of benzimidazoles in mouse bone marrow. *Mutat. Res.* 300:15-28.

Perocco, P., Del Ciello, C., Mazzullo, M., Rocchi, P., Ferreri, A.M., Paolini, M., Pozzetti, L. and Cantelli-Forti, G. (1997) Cytotoxicity and cell transforming activities of the fungicide methyl thiophanate on BALB/c 3T3 cells *in vitro*. *Mutat. Res.* 394:29-35.

Selling, H.A., Vonk, J.W. and Kaars Sijpesteijn, A. (1970) Transformation of the systemic fungicide methyl thiophanate into 2-benzimidazole carbamic acid methyl ester, in: *Chemistry and Industry*, Madley, London, pp. 1625-1626.

US EPA "Assessment of thyroid follicular cell tumors" (1998) Publication EPA/630/R-97/002, March, 1998.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T. and Mortelmans, K. (1992) *Salmonella* mutagenicity tests: IV Results of testing 311 chemicals. *Environ. And Molec. Mutagen* 19[Suppl.21]:2-141.

SignOff Date:
DP Barcode: D272850
HED DOC Number:
Toxicology Branch: RRB3